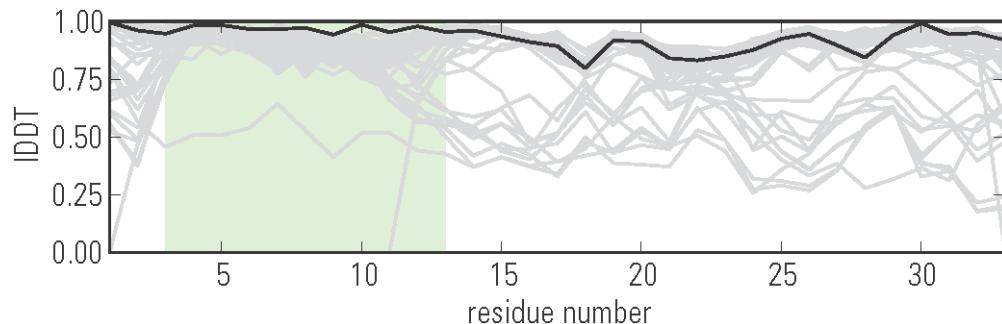


## **Supplementary Material**

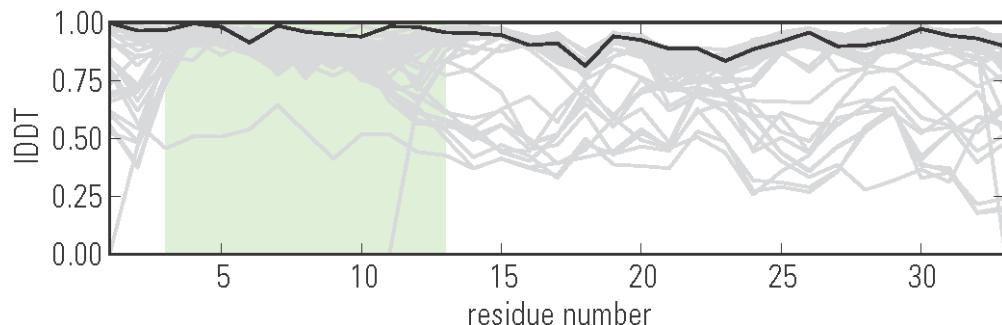
**Numerical assessment of engineered loops in targets  
T0709, T0711 and R0003.**

# T0709

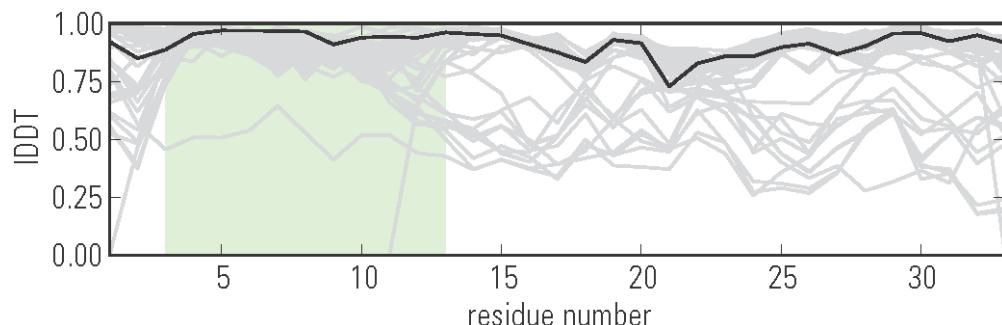
## A: Predictions by FALCON-TOPO-X



## B: Prediction by MUFold\_CRF



## C: Prediction by STRINGS



**Figure S1: Local accuracy assessment of target T0709.** The per-residue accuracy of predictions was evaluated using all-atom IDDT in multi-reference mode against the NMR ensemble (cut-off radius 10 Å, sequence separation of zero). The engineered loop region (residues 3-13) is shaded. Results by all groups are shown in grey with the best loop predictions highlighted in bold. See table S1 for a complete list of scores. Since the loop region shows significant deviations within the NMR ensemble, many loop predictions score comparably in the multi-reference IDDT analysis.

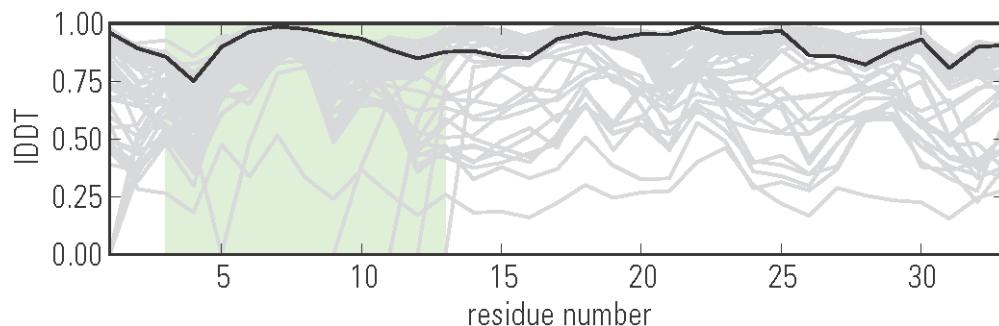
**Table S1: Assessment of the prediction accuracy of the engineered loop in target T0709.** The per-residue accuracy of predictions for the engineered loop (residues 3-13) was evaluated using IDDT in multi-reference mode against the NMR ensemble (cut-off radius 10A, sequence separation of zero). Values are provided for global model accuracy on C $\alpha$  (lddt\_ca) and all atoms (lddt\_aa) and only the loop region for C $\alpha$  and all-atoms (lddt\_loop\_ca, lddt\_loop\_aa), respectively. Table is sorted by lddt\_loop\_aa. Please note that this assessment of a single loop should be interpreted as a proof of principle, and has no statistical significance which would allow a meaningful ranking of methods.

Method	l <sub>ddt</sub> _ca	l <sub>ddt</sub> _aa	l <sub>ddt</sub> _loop_ca	l <sub>ddt</sub> _loop_aa
FALCON-TOPO-X	0.9329	0.9244	0.988545	0.966455
MUFold_CRF	0.9286	0.9292	0.977136	0.9643
STRINGS	0.9386	0.9081	0.973255	0.945618
hGen3D	0.9343	0.9113	0.973755	0.937173
sysimm	0.9357	0.9183	0.964927	0.935282
AOBA-server	0.9157	0.9203	0.908755	0.935136
MULTICOM-NOVEL	0.9357	0.923	0.966109	0.932418
MATRIX	0.9271	0.9044	0.959764	0.931182
QUARK	0.9443	0.9215	0.955409	0.929545
Zhang-Server	0.9414	0.9206	0.966991	0.923564
UGACSBL	0.9329	0.9164	0.954418	0.923118
PMS	0.9157	0.913	0.936873	0.920564
IntFOLD	0.9243	0.9077	0.935182	0.917973
samcha-server	0.9286	0.9107	0.937291	0.916682
BAKER-ROSETTASERVER	0.9429	0.9315	0.949673	0.916664
HHpredA	0.9343	0.9208	0.928918	0.915718
HHpredAQ	0.9343	0.9208	0.928918	0.915718
slbio	0.93	0.9173	0.935755	0.913809
Distill_roll	0.8714	0.8562	0.918764	0.910055
Mufold-MD	0.9229	0.9019	0.961564	0.909482
MUFOLD-Server	0.9229	0.9019	0.961564	0.9093
IntFOLD2	0.9271	0.9249	0.897827	0.908645
chunk-TASSER	0.9143	0.8934	0.921445	0.908073
BhageerathH	0.9343	0.9112	0.931809	0.906164
NewSerf	0.8757	0.8598	0.915064	0.906136
chuo-repack-server	0.9257	0.924	0.877945	0.906109
FALCON-TOPO	0.92	0.9072	0.936727	0.904982
ZHOU-SPARKS-X	0.9057	0.9133	0.891664	0.904473
MULTICOM-REFINE	0.9329	0.9194	0.924591	0.904064
RaptorX	0.9271	0.9263	0.917609	0.903645

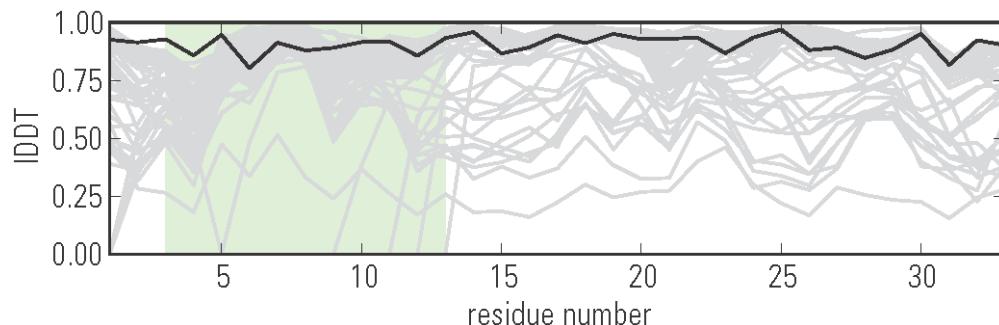
MULTICOM-CONSTRUCT	0.9286	0.9181	0.931	0.903327
Distill	0.9286	0.9052	0.929773	0.902891
GSmetaserver	0.92	0.9133	0.894118	0.9007
Seok-server	0.9343	0.9171	0.930864	0.9006
PconsM	0.9371	0.8769	0.960636	0.900555
FFAS03c	0.9343	0.9149	0.888809	0.900491
Pcons-net	0.93	0.9164	0.932327	0.898609
SAM-T08-server	0.9429	0.9188	0.977	0.897655
YASARA	0.9271	0.9103	0.9188	0.896464
PconsD	0.9386	0.882	0.953609	0.896227
RaptorX-ZY	0.9357	0.9185	0.927127	0.894673
MULTICOM-CLUSTER	0.9429	0.9221	0.935664	0.894473
Jiang_Fold	0.8957	0.8693	0.975027	0.892145
TASSER-VMT	0.92	0.8838	0.936227	0.890409
FFAS03hj	0.8743	0.8696	0.9237	0.887282
chuo-fams-server	0.9229	0.9106	0.8865	0.883527
HHpred-thread	0.9329	0.9138	0.927691	0.880736
Bilab-ENABLE	0.8714	0.86	0.9257	0.8806
3D-JIGSAW_V5-0	0.8371	0.8335	0.902973	0.877718
SAM-T06-server	0.9129	0.8968	0.912118	0.876055
Atome2_CBS	0.9186	0.9036	0.900918	0.872173
Phyre2_A	0.9014	0.8999	0.816764	0.867364
RBO-MBS-BB	0.7571	0.7268	0.879255	0.845645
Jiang_Threader	0.4471	0.4707	0.876527	0.833418
confuzz3d	0.6514	0.6413	0.879682	0.8307
Jiang_Server	0.4814	0.4887	0.859291	0.827
confuzzGS	0.5729	0.5785	0.857591	0.813436
RBO-i-MBS	0.5929	0.6068	0.825591	0.809755
RBO-MBS	0.6257	0.6409	0.856518	0.808009
FRESS_server	0.6586	0.6694	0.807409	0.807191
RBO-i-MBS-BB	0.59	0.6048	0.810927	0.804509
panther	0.4943	0.5121	0.833573	0.802173
Lenserver	0.7229	0.4496	0.922718	0.499736
FFAS03mt	0.7271	0.6971	0.136364	0.135345
FFAS03	0.7271	0.6945	0.133836	0.134018

# T0711

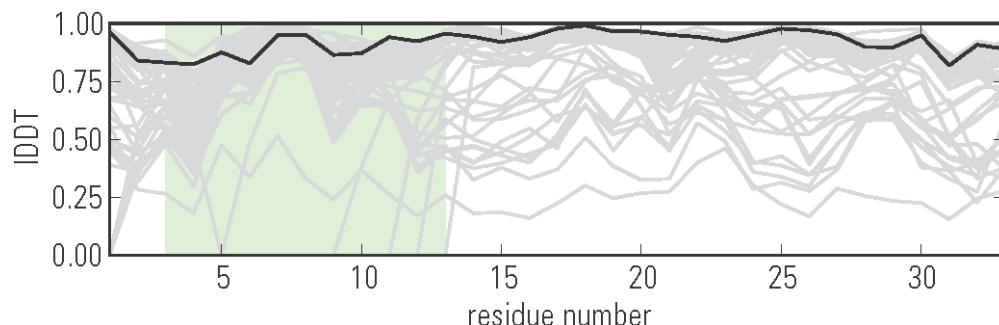
## A: Predictions by BAKER-ROSETTASERVER and PconsM



## B: Prediction by BhageerathH



## C: Prediction by MULTICOM-REFINE



**Figure S2: Local accuracy assessment of target T0711.** The per-residue accuracy of predictions was evaluated using all-atom IDDT in multi-reference mode against the NMR ensemble (cut-off radius 10 Å, sequence separation of zero). The engineered loop region (residues 3-13) is shaded. Results by all groups are shown in grey with the best loop predictions highlighted in bold. See table S2 for a complete list of scores.

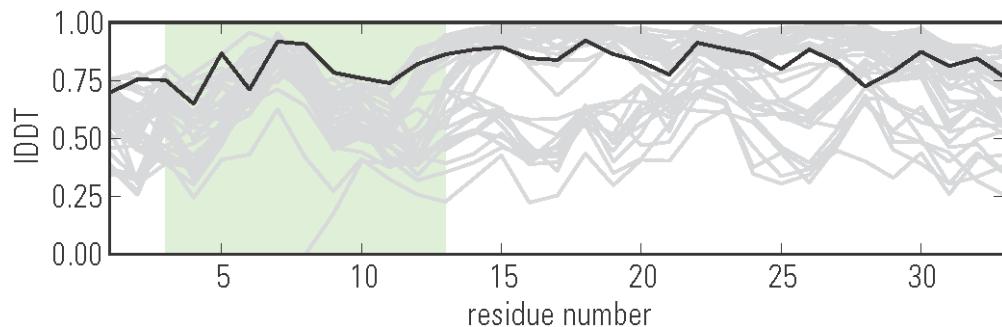
**Table S2: Assessment of the prediction accuracy of the engineered loop in target T0711.** The per-residue accuracy of predictions for the engineered loop (residues 3-13) was evaluated using IDDT in multi-reference mode against the NMR ensemble (cut-off radius 10A, sequence separation of zero). Values are provided for global model accuracy on C $\alpha$  (lddt\_ca) and all atoms (lddt\_aa) and only the loop region for C $\alpha$  and all-atoms (lddt\_loop\_ca, lddt\_loop\_aa), respectively. Table is sorted by lddt\_loop\_aa. Please note that this assessment of a single loop should be interpreted as a proof of principle, and has no statistical significance which would allow a meaningful ranking of methods.

Prediction	lddt_ca	lddt_aa	lddt_loop_ca	lddt_loop_aa
BAKER-ROSETTASERVER	0.9269	0.8972	0.931482	0.902355
PconsM	0.9269	0.8972	0.931482	0.902355
BhageerathH	0.9441	0.9037	0.933891	0.894327
MULTICOM-REFINE	0.9548	0.9189	0.914618	0.8921
Pcons-net	0.9162	0.8826	0.926264	0.889918
RaptorX-ZY	0.9574	0.9253	0.929445	0.888091
PconsD	0.9654	0.8762	0.970582	0.883055
MULTICOM-NOVEL	0.9495	0.916	0.904955	0.880818
IntFOLD2	0.9548	0.8987	0.967255	0.877845
MULTICOM-CONSTRUCT	0.9428	0.9111	0.897364	0.876145
HHpredA	0.9428	0.909	0.893191	0.874018
HHpredAQ	0.9428	0.909	0.893191	0.874018
UGACSBL	0.9508	0.9036	0.933182	0.871409
Seok-server	0.9495	0.9073	0.904491	0.867736
RaptorX	0.9468	0.905	0.899691	0.864882
chuo-fams-server	0.9481	0.8981	0.899882	0.854555
Atome2_CBS	0.9481	0.9065	0.9182	0.853764
PMS	0.9255	0.8905	0.881336	0.852591
MULTICOM-CLUSTER	0.9508	0.9056	0.899127	0.8519
chuo-repack-server	0.9495	0.9103	0.909891	0.850964
PROTAGORAS	0.9428	0.9	0.882491	0.845509
hGen3D	0.9481	0.8861	0.897336	0.840473
ZHOU-SPARKS-X	0.9428	0.8811	0.902718	0.840227
Bilab-ENABLE	0.867	0.8386	0.870364	0.839655
QUARK	0.9295	0.8908	0.871336	0.836882
SAM-T08-server	0.9269	0.883	0.892055	0.834045
Phyre2_A	0.9574	0.8983	0.933282	0.827045
HHpred-thread	0.9388	0.8956	0.843436	0.825564
FRESS_server	0.8763	0.8329	0.872727	0.822555
Zhang-Server	0.9402	0.8735	0.892682	0.821173
SAM-T06-server	0.9176	0.8848	0.840945	0.818227
MUFOLD-Server	0.9016	0.8632	0.8907	0.817473
chunk-TASSER	0.9255	0.8612	0.877736	0.815791

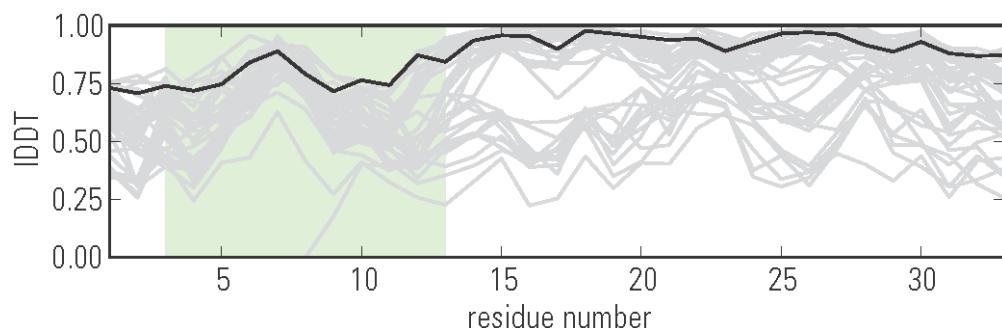
samcha-server	0.9109	0.8692	0.8534	0.809755
RBO-MBS	0.6875	0.6593	0.878364	0.807982
TASSER-VMT	0.9441	0.8771	0.896136	0.806555
IntFOLD	0.8843	0.8288	0.8668	0.805427
YASARA	0.9362	0.8803	0.874491	0.797645
slbio	0.8604	0.8353	0.811027	0.781891
RBO-MBS-BB	0.6862	0.6602	0.845491	0.781055
3D-JIGSAW_V5-0	0.6223	0.6479	0.816018	0.777473
FALCON-TOPO-X	0.8657	0.8289	0.825691	0.775382
FALCON-TOPO	0.8777	0.8349	0.855945	0.768391
AOBA-server	0.8684	0.8215	0.825945	0.757618
STRINGS	0.8963	0.8353	0.784118	0.756664
Jiang_Fold	0.891	0.8385	0.781936	0.755255
confuzz3d	0.5864	0.5585	0.836764	0.738018
NewSerf	0.8843	0.8325	0.775491	0.7348
confuzzGS	0.5838	0.57	0.793273	0.7326
Mufold-MD	0.8896	0.8443	0.766191	0.729864
Distill_roll	0.9056	0.8312	0.799845	0.728382
Distill	0.8816	0.8004	0.765045	0.700618
MATRIX	0.8856	0.811	0.782509	0.690873
RBO-i-MBS-BB	0.6316	0.6091	0.712218	0.673136
RBO-i-MBS	0.6396	0.6121	0.723136	0.671891
Bhageerath_ab initio	0.5266	0.4977	0.702155	0.634636
Jiang_Threader	0.4229	0.4274	0.676982	0.622264
MUFold_CRF	0.7739	0.743	0.519082	0.535082
HOMER	0.8112	0.7414	0.366345	0.347355
Jiang_Server	0.6024	0.2514	0.797282	0.309455
FFAS03mt	0.7021	0.6456	0.141191	0.128573
FFAS03	0.6955	0.6409	0.141191	0.1276
FFAS03c	0.6955	0.6409	0.141191	0.1276
FFAS03hj	0.6476	0.5934	0.073864	0.065309
sysimm	0.5824	0.5163	0	0

# R0003

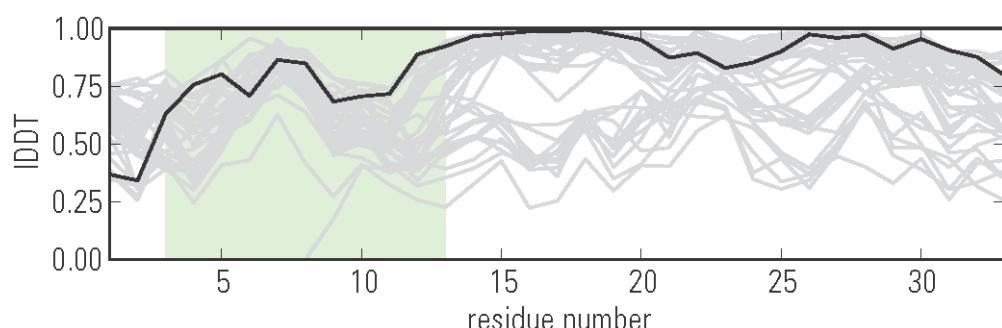
## A: Predictions by MidwayFolding



## B: Prediction by BhageerathH



## C: Prediction by SAM-T08-server



**Figure S3: Local accuracy assessment of target R0003.** The per-residue accuracy of predictions was evaluated using all-atom IDDT in multi-reference mode against the NMR ensemble (cut-off radius 10 Å, sequence separation of zero). The engineered loop region (residues 3-13) is shaded. Results by all groups are shown in grey with the best loop predictions highlighted in bold. See table S3 for a complete list of scores.

**Table S3: Assessment of the prediction accuracy of the engineered loop in target R0003.** The per-residue accuracy of predictions for the engineered loop (residues 3-13) was evaluated using IDDT in multi-reference mode against the NMR ensemble (cut-off radius 10A, sequence separation of zero). Values are provided for global model accuracy on C $\alpha$  (lddt\_ca) and all atoms (lddt\_aa) and only the loop region for C $\alpha$  and all-atoms (lddt\_loop\_ca, lddt\_loop\_aa), respectively. Table is sorted by lddt\_loop\_aa. Please note that this assessment of a single loop should be interpreted as a proof of principle, and has no statistical significance which would allow a meaningful ranking of methods.

Prediction	lDDT_ca	lDDT_aa	lDDT_loop_ca	lDDT_loop_aa
MidwayFolding	0.8614	0.8174	0.872518	0.796945
BhageerathH	0.9148	0.873	0.852009	0.787245
SAM-T08-server	0.8636	0.8558	0.816427	0.774864
PconsQ	0.8659	0.8489	0.817364	0.764082
MULTICOM-CLUSTER	0.9023	0.8753	0.806182	0.748155
SAM-T06-server	0.8659	0.8305	0.814282	0.743645
SHORTLE	0.5773	0.5534	0.751382	0.735391
BAKER	0.8909	0.854	0.770273	0.719109
chunk-TASSER	0.6761	0.6224	0.731409	0.718891
CphBeta	0.8636	0.8407	0.753009	0.717673
keasar	0.8875	0.8538	0.763664	0.712
PconsD	0.7807	0.7629	0.778745	0.708264
Jones-UCL	0.6523	0.6173	0.735136	0.693309
zhang	0.6705	0.6371	0.703682	0.680745
MULTICOM-NOVEL	0.8318	0.803	0.717209	0.676427
Zhang_Ab_Initio	0.6727	0.6314	0.695764	0.663909
MBBS	0.817	0.7996	0.685609	0.653709
nns	0.8148	0.7986	0.680809	0.650018
LEE	0.8148	0.7986	0.680809	0.650018
TsaiLab	0.7852	0.774	0.671982	0.649691
MULTICOM	0.8193	0.7813	0.6764	0.648927
ZHOU-SPARKS-X	0.8136	0.796	0.623136	0.646873
MULTICOM-CONSTRUCT	0.8205	0.7966	0.676073	0.646655
QUARK	0.8455	0.8007	0.683109	0.642073
Zhang-Server	0.85	0.7857	0.688164	0.641036
Seok-server	0.8136	0.7804	0.667391	0.638936
Seok	0.8205	0.7984	0.675545	0.635718
Pcons	0.8261	0.7974	0.666564	0.632373
MidwayFoldingHuman	0.8136	0.7898	0.660109	0.630982
ossia	0.858	0.8249	0.662655	0.626736
MULTICOM-REFINE	0.8205	0.782	0.676309	0.625391
Pcons-net	0.7557	0.7376	0.643891	0.623755
BAKER-ROSETTASERVER	0.6466	0.5994	0.648882	0.617709
TASSER	0.8273	0.771	0.653082	0.6143

Contenders	0.6705	0.6193	0.627673	0.612209
Bilab	0.5989	0.5628	0.638727	0.601255
Cornell-Gdansk	0.508	0.497	0.598773	0.594809
Distill_roll	0.8148	0.7448	0.616082	0.587427
Anthropic_Dreams	0.5193	0.4934	0.571636	0.566655
FOLDIT	0.775	0.7322	0.586082	0.559373
Void_Crushers	0.7659	0.7232	0.575036	0.552245
Laufer	0.4977	0.4694	0.5003	0.501518
ProQ2	0.6239	0.3617	0.671873	0.366973
FALCON-Server	0.6239	0.3617	0.671873	0.366973
panther	0.6045	0.5666	0.184009	0.181791